

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Original) A process for extracting native or recombinantly-expressed, gram-negative inner membrane proteins from bacteria or bacterial host cells containing a recombinant vector by differential detergent tangential flow diafiltration, which comprises:
 - (a) lysing bacteria or bacterial host cells containing a recombinant vector in a fermentation broth;
 - (b) diafiltering the lysed fermentation broth from (a) with a buffer which is not retained by the diafiltration membrane, wherein said buffer removes intracellular and extracellular contaminants through the permeate, and using a chelating agent to prevent proteolysis;
 - (c) diafiltering the lysate from (b) with a detergent and a buffer which is not retained by the diafiltration membrane, wherein said detergent solubilizes and removes inner membrane proteins, and using a divalent cation to stabilize the outer membrane proteins, thereby preventing their solubilization; and
 - (d) collecting the inner membrane proteins removed in (c).
2. (Currently amended) The process of Claim 1 wherein: the lysis of (a) occurs in a microfluidizer; in (b), the buffer is selected from the group consisting of Hepes, 3-(N-morpholino)propane sulfonic acid (MOPS), Tris(hydroxymethyl)aminomethane Tris™, sodium phosphate and sodium borate; and in (c), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane Tris™, sodium phosphate and sodium borate, the detergent is selected from the group consisting of a zwitterionic detergent Zwittergent™ compound, a non-ionic detergent Triton™ compound, sarcosyl, a glucoside compound, a cholate compound and dodecyl-

maltoside, and the divalent cation is selected from the group consisting of magnesium and calcium (Mg^{+2} and Ca^{+2}).

3. (Currently amended) The process of Claim 2 wherein in (b), the buffer is Hepes and the chelating agent is EDTA; and in (c), the buffer is Hepes, the detergent is alpha-[4-(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl) Triton™ X-100, and the divalent cation is Mg^{+2} .

4. (Original) A process for extracting native or recombinantly-expressed, gram-negative outer membrane proteins from bacteria or bacterial host cells containing a recombinant vector by differential detergent tangential flow diafiltration, which comprises:

- (a) lysing bacteria or bacterial host cells containing a recombinant vector in a fermentation broth;
- (b) diafiltering the lysed fermentation broth from (a) with a buffer which is not retained by the diafiltration membrane, wherein said buffer removes intracellular and extracellular contaminants through the permeate, and using a chelating agent to prevent proteolysis;
- (c) diafiltering the lysate from (b) with a detergent and a buffer which is not retained by the diafiltration membrane, wherein said detergent solubilizes and removes inner membrane proteins, and using a divalent cation to stabilize the outer membrane proteins, thereby preventing their solubilization;
- (d) diafiltering the lysate from (c) with the buffer from (c), and using a divalent cation from (c) in the absence of detergent, in order to reduce the concentration of the detergent from (c);
- (e) diafiltering the lysate from (d) with a buffer which is not retained by the diafiltration membrane, a chelating agent and a detergent to solubilize and remove the outer membrane proteins; and
- (f) collecting the outer membrane proteins removed in (e).

5. (Currently amended) The process of Claim 4 wherein: the lysis of (a) occurs in a microfluidizer; in (b), the buffer is selected from the group consisting of Hepes,

MOPS, Tris(hydroxymethyl)aminomethane TrisTM, sodium phosphate and sodium borate; in (c), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane TrisTM, sodium phosphate and sodium borate, the detergent is selected from the group consisting of a zwitterionic detergent ZwittergentTM compound, a non-ionic detergent TritonTM com, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside, and the divalent cation is selected from the group consisting of Mg⁺² and Ca⁺²; in (d), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane TrisTM, sodium phosphate and sodium borate, and the divalent cation is selected from the group consisting of Mg⁺² and Ca⁺²; and in (e), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane TrisTM, sodium phosphate and sodium borate, and the detergent is selected from the group consisting of a zwitterionic detergent ZwittergentTM, a non-ionic detergent TritonTM com, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside.

6. (Currently amended) The process of Claim 3 wherein in (b), the buffer is Hepes and the chelating agent is EDTA; in (c), the buffer is Hepes, the detergent is alpha-[4-(1,1,3,3,-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl) TritonTM X-100, and the divalent cation is Mg⁺²; in (d), the buffer is Hepes and the divalent cation is Mg⁺²; and in (e), the buffer is Tris(hydroxymethyl)aminomethane TrisTM, the chelating agent is EDTA, and the detergent is n-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate ZwittergentTM 3-12.

7. (Original) The process of Claim 4, which further comprises:

- (g) diafiltering the lysate from (e) with reagents of (e), with the exception of the detergent, in order to reduce the concentration of the detergent;
- (h) diafiltering the lysate from (g) with reagents of (e); and
- (i) collecting the outer membrane proteins removed in (h).

8. (Original) A process for extracting lipidated recombinant outer membrane protein P4 (rP4) of *Haemophilus influenzae* from bacterial host cells by differential detergent tangential flow diafiltration, which comprises:

- (a) lysing bacterial host cells in a fermentation broth;
- (b) diafiltering the lysed fermentation broth from (a) with a buffer which is not retained by the diafiltration membrane, wherein said buffer removes intracellular and extracellular contaminants through the permeate, and using a chelating agent to prevent proteolysis;
- (c) diafiltering the lysate from (b) with a detergent and a buffer which is not retained by the diafiltration membrane, wherein said detergent solubilizes and removes inner membrane proteins, and using a divalent cation to stabilize the outer membrane proteins, thereby preventing their solubilization;
- (d) diafiltering the lysate from (c) with the buffer from (c), and using a divalent cation from (c) in the absence of detergent, in order to reduce the concentration of the detergent from (c);
- (e) diafiltering the lysate from (d) with a buffer which is not retained by the diafiltration membrane, a chelating agent and a detergent to solubilize the outer membrane proteins;
- (f) diafiltering the lysate from (e) with a buffer which is not retained by the diafiltration membrane, a chelating agent and a detergent to extract and remove the lipidated rP4; and
- (g) collecting the lipidated rP4 removed in (f).

9. (Currently amended) The process of Claim 8 wherein: the lysis of (a) occurs in a microfluidizer; in (b), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane Tris™, sodium phosphate and sodium borate; in (c), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane Tris™, sodium phosphate and sodium borate, the detergent is selected from the group consisting of a zwitterionic detergent Zwittergent™, a non-ionic detergent Triton™ com, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside, and the divalent cation is selected from the group consisting of Mg^{+2} and Ca^{+2} ; in (d), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane Tris™, sodium phosphate and sodium borate, and the divalent cation is selected from the group consisting of Mg^{+2} and Ca^{+2} ; in (e), the buffer is selected from the group consisting of Hepes, MOPS,

Tris(hydroxymethyl)aminomethane Tris™, sodium phosphate and sodium borate, and the detergent is selected from the group consisting of a zwitterionic detergent Zwittergent™, a non-ionic detergent Triton™-com, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside; and in (f), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane Tris™, sodium phosphate and sodium borate, and the detergent is selected from the group consisting of a zwitterionic detergent Zwittergent™, a non-ionic detergent Triton™-com compound, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside.

10. (Currently amended) The process of Claim 9 wherein in (b), the buffer is Hepes and the chelating agent is EDTA; in (c), the buffer is Hepes, the detergent is alpha-[4-(1,1,3,3,-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl) Triton™ X-100, and the divalent cation is Mg⁺²; in (d), the buffer is Hepes and the divalent cation is Mg⁺²; in (e), the buffer is Tris(hydroxymethyl)aminomethane Tris™, the chelating agent is EDTA, and the detergent is n-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate Zwittergent™ 3-12; and in (f), the buffer is Tris(hydroxymethyl)aminomethane Tris™, the chelating agent is EDTA, and the detergent is n-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate Zwittergent™ 3-12.

11. (Original) The process of Claim 8, which further comprises:

- (h) diafiltering the lysate from (f) with reagents of (f), with the exception of the detergent, in order to reduce the concentration of the detergent;
- (i) diafiltering the lysate from (h) with reagents of (f) to extract and remove the lipidated rP4; and
- (j) collecting the lipidated rP4 removed in (i).

12. (Original) The process of Claim 11, which further comprises:

- (k) diafiltering the lysate from (j) with reagents of (f), with the exception of the detergent, in order to reduce the concentration of the detergent;
- (l) diafiltering the lysate from (k) with reagents of (f) to extract and remove the lipidated rP4; and
- (m) collecting the lipidated rP4 removed in (l).

13. (Original) A process for extracting lipidated recombinant outer membrane protein P6 (rP6) of *Haemophilus influenzae* from bacterial host cells by differential detergent tangential flow diafiltration, which comprises:

- (a) lysing bacterial host cells in a fermentation broth;
- (b) diafiltering the lysed fermentation broth from (a) with a buffer which is not retained by the diafiltration membrane, wherein said buffer removes intracellular and extracellular contaminants through the permeate, and using a chelating agent to prevent proteolysis;
- (c) diafiltering the lysate from (b) with a detergent and a buffer which is not retained by the diafiltration membrane, wherein said detergent solubilizes and removes inner membrane proteins, and using a divalent cation to stabilize the outer membrane proteins, thereby preventing their solubilization;
- (d) diafiltering the lysate from (c) with a buffer which is not retained by the diafiltration membrane, a chelating agent to sequester divalent cation and to prevent proteolysis, and a detergent to solubilize and remove the outer membrane proteins other than lipidated rP6;
- (e) diafiltering the lysate from (d) with a buffer which is not retained by the diafiltration membrane, a chelating agent to prevent proteolysis, a detergent to remove additional outer membrane proteins, and a salt to disrupt the membrane/outer membrane protein complex;
- (f) diafiltering the lysate from (e) with reagents of (e), with the exception of the detergent and the salt, in order to reduce the concentration of the detergent;
- (g) diafiltering the lysate from (f) with a detergent and a buffer which is not retained by the diafiltration membrane, wherein said detergent solubilizes and removes additional proteins bound to the cellular outer membrane, and using a chelating agent to prevent proteolysis;
- (h) diafiltering the lysate from (g) with the buffer from (g) and the chelating agent of (g) to reduce the concentration of the detergent from (g);
- (i) diafiltering the lysate from (h) with a phosphate compound and a detergent to solubilize and remove additional proteins bound to the cellular outer membrane;

- (j) diafiltering the lysate from (i) with a phosphate compound to reduce the concentration of the detergent from (i);
- (k) heating the lysate from (j) to remove lipidated rP6 from the membrane while diafiltering that lysate with a phosphate compound and a detergent to solubilize, extract and remove the lipidated rP6; and
- (l) collecting the lipidated rP6 removed in (k).

14. (Currently amended) The process of Claim 13 wherein: the lysis of (a) occurs in a microfluidizer; in (b), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane Tris™, sodium phosphate and sodium borate, and the divalent cation is selected from the group consisting of Mg^{+2} and Ca^{+2} ; in (c), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane Tris™, sodium phosphate and sodium borate, the detergent is selected from the group consisting of a zwitterionic detergent Zwittergent™, a non-ionic detergent Triton™ com, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside, and the divalent cation is selected from the group consisting of Mg^{+2} and Ca^{+2} ; in (d), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane Tris™, sodium phosphate and sodium borate, and the detergent is selected from the group consisting of a zwitterionic detergent Zwittergent™, a non-ionic detergent Triton™ com, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside; in (e), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane Tris™, sodium phosphate and sodium borate, the salt is a sodium salt, and the detergent is selected from the group consisting of a zwitterionic detergent Zwittergent™, a non-ionic detergent Triton™ com, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside; in (f), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane Tris™, sodium phosphate and sodium borate; in (g), the buffer is selected from the group consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane Tris™, sodium phosphate and sodium borate, and the detergent is selected from the group consisting of a zwitterionic detergent Zwittergent™, a non-ionic detergent Triton™ com, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside; in (h), the buffer is selected from the group

consisting of Hepes, MOPS, Tris(hydroxymethyl)aminomethane Tris™, sodium phosphate and sodium borate; in (i), the detergent is selected from the group consisting of a zwitterionic detergent Zwittergent™, a non-ionic detergent Triton™-com, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside; and in (k), the detergent is selected from the group consisting of a zwitterionic detergent Zwittergent™, a non-ionic detergent Triton™-com, sarcosyl, a glucoside compound, a cholate compound and dodecyl-maltoside.

15. (Currently amended) The process of Claim 14 wherein in (b), the buffer is Hepes and the chelating agent is EDTA; in (c), the buffer is Hepes, the detergent is alpha-[4-(1,1,3,3-tetramethylbutyl)phenyl]-omega-hydroxypoly(oxy-1,2-ethanediyl) Triton™-X-100, and the divalent cation is Mg^{+2} ; in (d), the buffer is Hepes, the chelating agent is EDTA, and the detergent is n-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate Zwittergent™-3-14; in (e), the buffer is Hepes, the chelating agent is EDTA, the salt is sodium chloride, and the detergent is n-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate Zwittergent™-3-14; in (f), the buffer is Tris(hydroxymethyl)aminomethane Tris™ and the chelating agent is EDTA; in (g), the buffer is Tris(hydroxymethyl)aminomethane Tris™, the detergent is sarcosyl, and the chelating agent is EDTA; in (h), the buffer is Tris(hydroxymethyl)aminomethane Tris™ and the chelating agent is EDTA; in (i), the detergent is n-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate Zwittergent™-3-12, and the phosphate is sodium phosphate; in (j), the phosphate is sodium phosphate; and in (k), the detergent is n-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate Zwittergent™-3-12.

16. (Original) The process of Claim 13 wherein prior to (k), the lysate from (j) is concentrated.